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Natural Sciences

HIGHLIGHT

A breakthrough from 60 years ago: "General nature of the genetic code for proteins" (1961)

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Abstract

In 1961, Francis Crick and Sydney Brenner, together with two Cambridge colleagues, published an article in *Nature* that used simple genetic experiments to demonstrate that the genetic code was almost certainly based on groups of three nucleotides. Six decades later, this article continues to be an inspiration to scientists due to its elegant argumentation and its use of simple, powerful experimentation to reveal fundamental truths about the organisation of living matter. This essay explores how and why the research was carried out, showing how the aims of the experiment gradually changed over time, and highlighting how the intense intellectual interactions between Crick and Brenner contributed to this model of scientific endeavour.

KEYWORDS
Francis Crick, Genetic code, Sydney Brenner, triplet code

On 30 December 1961, a paper was published in *Nature* that became an instant classic of molecular biology [1]. It combined theory and experimentation in a striking display of the incisive thinking of the first author, Francis Crick, and of his intensely productive interactions with his friend, colleague and co-author, Sydney Brenner [2]. Simply using the power of genetics, Crick, Brenner and their colleagues showed that the genetic code was almost certainly based on groups of three nucleotides, long before it was possible to sequence nucleic acids.

Although grasping all the experimental detail in the paper was (and is) complex for the uninitiated reader, the overall effect was so significant that papers and books have repeatedly summarised its key findings in detail and the paper has been cited over 900 times [3,4,5,6]. It has been described as "one of the most remarkable papers in biology" [7], and its continuing influence is as much due to its style and rigour as to its historical place in the development of molecular biology.

The background to the paper, both in terms of the context in which the work was done, and the informal networks that surrounded it, shed light on this key moment in the history of science, and show what has changed in how we carry out our research – and what has remained the same. The work around the creation of the paper also reveals something that we all know to be true – scientific articles are generally constructs, retrospectively presented to explain and justify a particular set of findings. Although the intellectual power of the article comes from the clarity of the theoretical statements and supporting data, all of which are focused on the central hypotheses outlined at the beginning, in reality the meaning of the results, and the decision on what was the next experiment to be done, emerged out of Crick and Brenner's tussles with the data. The end point of their experimental journey became gradually clearer as they took each step, but it was neither evident nor determined at the outset. For all its elegance, the paper represents an intellectual argument that gelled only towards the end of proceedings, not a diary of experiments that were carried out sequentially and according to a pre-determined plan. As Crick later admitted, "I don't think I could honestly call it 'logically planned'. I think I'd call it 'logically improvised'" [8].

1 | BACKGROUND

Entitled "General nature of the genetic code for proteins", the article was the culmination of a series of breakthroughs in our understanding of gene function that were made in 1961, transforming our view of what genes do and how they do it:

- In May, *Nature* published back-to-back articles from Sydney Brenner, François Jacob and Matthew Meselson on the one hand [9], and from a group led by Jim Watson on the other [10]. Using different methods, both papers described the existence of messenger RNA that played an intermediate role between DNA and the ribosome, the site where proteins are assembled out of amino acids [11].
- Also in May, François Jacob and Jacques Monod shaped our understanding of what genes do, when in an article in *Journal of Molecular Biology* they classified genes as either playing a structural role – encoding a protein – or a regulatory role, performing "operations which control the rate of transfer of structural information from gene to protein" [12].
- In August 1961, Marshall Nirenberg, an unknown NIH researcher, announced the solution to a problem that had defeated some of the greatest minds of biology, mathematics and physics – he had cracked the genetic code. Taking an experimental approach, Nirenberg used a cell-free test-tube system to synthesise the amino acid phenylalanine using a stretch of RNA composed of only one base, uracil. In the autumn, this discovery appeared in the shape of two articles in the *Proceedings of the National Academy of Sciences* [13,14].

Although Nirenberg's experiment – carried out with Heinrich Matthaei – marked a massive breakthrough, the general nature of the genetic code – how many bases corresponded to each amino acid – remained unclear. Crick and Brenner had been thinking about this issue for some time; 5 years earlier, Brenner had shown that the genetic code could not be overlapping, that is, the genetic 'words', composed of nucleotides, must be discreet and read in order [15].

Some simple arithmetic had long convinced scientists that it was probable that each genetic "word" was composed of three nucleotides. (We now call this unit a "codon", but that term had not yet been coined by Brenner; it was first used in public by Crick in 1962 [16].) The argument was as follows: the informational part of a DNA molecule consists of four kinds of nucleotide – the bases A, C, G and T – while there were 20 naturally occurring amino acids. If the genetic code used groups of two bases, there would only be 16 combinations; however, if the code were based on three bases, there were 64 combinations. This higher number (based on the general formula r^n, the number of strings of length n that can be formed from a set of r characters) raised substantial problems. While it easily covered what Crick called "the magic number 20" [17], it implied either that many of the 64 combinations did not code for anything and were ignored by the cell, or that the code was, in the terminology of the time, "degenerate", with several combinations encoding each amino acid. That possibility seemed wasteful and clunky, and was rejected by many of the theoreticians who dwelt on this problem, and were influenced by a mathematical predilection for parsimony and elegance.

In Spring 1961, Crick and Brenner were trying to experimentally test a now-forgotten theory of the genetic code which they called "loopy codes". They used a virus known as T4 – this was a bacteriophage or "phage"; it was the workhorse of the early decades of molecular biology and had been intensively explored by Crick and Brenner's close friend Seymour Benzer while he was on a sabbatical at Cambridge a few years earlier. Benzer had mapped the rII genetic region of T4 through a series of incredibly detailed crosses between different T4 lines and had shown that mutations in some parts of the genome rendered the virus non-functional, unable to infect bacteria [18].

Loopy codes rapidly turned out to be a dead end, but Crick had enjoyed working in the laboratory (this was not something he did often, mainly because he was renowned as a poor experimentalist – veteran Cambridge technician Muriel Wigby described him as "terribly clumsy"[19]). During his otherwise fruitless exploration, Crick had observed a surprisingly large number of T4 mutations that suppressed the effect of other mutations, in particular, by altering the behaviour of strains that otherwise could not infect certain bacteria. After one of their many interminable "mad sessions" in which they threw ideas about pell-mell, Crick and Brenner decided to explore this phenomenon in greater detail.

Crick and Brenner's starting point was quite simple and relatively unambitious. Brenner had shown that certain dyes known as acridines could induce a mutation in a single base in the T4 virus. Up until this point, chemical mutagens had transformed one base into another, but acridine dyes could apparently either add or subtract bases – this novel feature was to prove decisive in what followed. In a brief paper written the previous autumn, Crick and Brenner, along with their colleague Leslie Orgel and his wife Alice Orgel, a PhD student, argued that deleting or adding a single base using an acridine dye would alter how the genetic information was read [20]. It could potentially render the message after the mutation non-sensical because what they eventually termed the "reading frame" (they initially called such mutations "phase-shift" mutants) would now be altered. For example, if there were a triplet code sequence such as ATG CAT CCC TGA … and the first C were deleted, then the sequence would become ATG ATC CCT GA … The first codon would be the same but the remaining codons would be altered. As Crick put it in their 1961 *Nature* article:

> "The simplest postulate to make is that the shift of the reading frame produces some triplets the reading of which is 'unacceptable'; for example, they may be 'nonsense', or stand for 'end the chain', or be unacceptable in some other way to the complications of protein structure" [21].

They would be able to tell if the virus carried a mutation not by sequencing the DNA – that lay over 15 years in the future – but by using the simplest of procedures: observing whether a particular viral strain could infect bacteria. Crick later described what it involved:

> "Phage genetics has the advantage that experiments are rather fast, once everything is set up. It does not take long to carry out a hundred crosses, since the manipulations are easy and an actual cross takes only about twenty minutes, this being the time for the phase to infect the bacterium, to multiply inside it (exchanging genetic material in the process), and to burst open, thus killing the cell. The results of the cross must then be plated out on petri dishes, to which a thin film of bacteria has been added. Then the dishes have to be incubated, to produce a lawn of bacteria. Where a single phage has landed and infected a cell, a colony of phage will grow, killing the local bacteria as it does so, forming a clear little hole (called a plaque) in the lawn of growing bacteria on the surface of the plate. (…) Then the petri dishes have to be taken from the 37°C incubator and examined to see whether they have plaques or not and, if so, of what type. Interesting plaques are then 'picked' – that is, a few phage are picked up with a little piece of paper or a toothpick; grown further; and the process repeated a second time to make sure the phage stock is a pure one" [22].

The simplicity of the experimental method employed in this study partly explains why so many researchers have found the article so impressive and attractive. There were no fancy pieces of equipment, no incomprehensible statistical analyses, no overly complex control experiments. Instead, it merely involved a researcher, some petri dishes and a lab book in which to record the results of crosses that were intellectually complex to conceive of, but which were remarkably simple to carry out.

2 | THE ROLE OF SUPPRESSORS

The key phase of the experiment began in May 1961 [23]. One weekend when no one else was in the laboratory, Crick was studying an acridine mutant that destroyed the ability of viruses to infect the K strain of *E. coli* bacteria (the mutant could still infect the B strain). He needed to give the mutant viral strain a number, but could not remember what letters had already been used in the lab's labelling system. So he called it "FC0" ("Francis Crick 0"), not because he was conceited, he later insisted, but because he had a very poor memory [24]. Using FC0 as his base strain, Crick began to look for mutants that would revert the strain back to wild-type, or 'suppress' the mutation.

Crick was not the only person to be thinking this way. In an example of how physicists were interested in the genetic code at the time, Richard Feynman had also been doing experiments on phage, in the Caltech laboratory of Max Delbrück. Feynman, too, had stumbled upon a suppressor mutation, but neither he nor Delbrück could find a satisfactory explanation. Crick got wind of Feynman's work and in June 1961 wrote a brief note to Delbrück asking what exactly had been discovered, while cryptically stating "We have an ingenious theory for our results that, if true, would be very important for decoding, but it needs much more work to establish it" [25].

The "ingenious theory" was that the original FC0 acridine mutation, which removed the ability to infect K strains of *E. coli*, had added a nucleotide to the viral genome, resulting in a sequence that had no meaning after that point. However, the deletion of a base close to the original mutation would restore the reading frame, restoring the function of the protein and suppressing the mutation (see Figure 1). Even if the resultant protein differed by a few amino acids compared to the wild-type, this might not be enough to affect viral function.

As the team emphasised repeatedly, they had no evidence that the original FC0 mutation had actually added a nucleotide, nor that the suppressor had deleted a base. They had no way of knowing, and it was equally possible that things had occurred the other way round (in other words, that FC0 was a deletion). From the point of view of the experiment, it did not matter – mutations and their interactions could be classified according to their effect on the original FC0 mutation.

After a highly productive few weeks in the laboratory, Crick had to stop work due to a number of obligations, both scientific and domestic. In June he went to a French conference on DNA, held at Col de Voz in the Alps, where he briefly described his experiments and made the following suggestion, which he subsequently gave to the organisers in written form, so it could be published in the conference proceedings:

> "the code is read in short groups, starting from one end of the gene. The exact starting point is supposed to determine which group is read. The deletion of a base would then alter the active reading from this point onward. The double mutants produced by the reversion of acridine mutants would then, on this hypothesis be altered not just in two, separated amino acids, but in a short stretch of amino acids in sequence" [26].

After the conference was over, Crick and his family travelled on to Tangier for a month-long holiday; Crick then flew to Moscow, to take part in the International Congress of Biochemistry in the second week of August.

Meanwhile, back in Cambridge, Brenner, microbiologist Leslie Barnett and physicist Richard Watts-Tobin, who was at the Laboratory of Molecular Biology working on acridine induction of mutations, were hard at work. Brenner kept Crick up to date by letter, describing their progress – or lack of it. On 27 July 1961, Brenner wrote to Crick in Morocco complaining that their attempts to map the suppressors were not making much sense – "the order seems to jump about quite a bit," he wrote [27]. Some of the mutations appeared to be located at the same site; rather than thinking of the effect of the mutations as adding or deleting a base, Brenner used the term "spin" as a neutral way of describing their nature. Referring to Barnett's work, he wrote:

> "She is also busy testing what I call the 'spin' of suppressors that map at the same site. This should be known by the end of next weekend is [sic] an important experiment; to test whether suppressors of opposite spin can nevertheless occupy the same site".

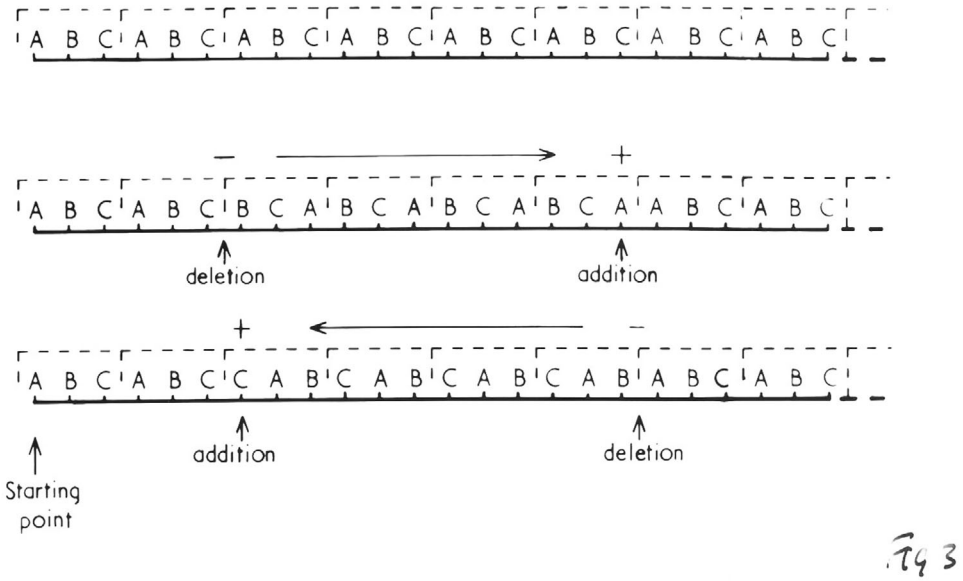


FIGURE 1 Figure 3 from the manuscript of Crick et al. (1961) showing the "ingenious theory" that lay behind the logic of the experiment. The published caption read: "To show that our convention for arrows is consistent. The letters *A*, *B* and *C* each represent a different base of the nucleic acid. For simplicity a repeating sequence of bases, *ABC*, is shown. (This would code for a polypeptide for which every amino-acid was the same.) A triplet code is assumed. The dotted lines represent the imaginary 'reading frame' implying that the sequence is read in sets of three starting on the left." Wellcome Collection. Attribution-NonCommercial 4.0 International (CC BY-NC 4.0)

Meanwhile, Crick was in Moscow, where, in a small room containing barely two dozen people, Marshall Nirenberg presented his bombshell experiment in which he described how he had cracked the genetic code. Crick heard the news from Matthew Meselson, who later recalled:

> "I heard the talk. And I was bowled over by it. … I went and chased down Francis, and told him that he must have a private talk with this man" [28].

Crick was amazed by Nirenberg's breakthrough – he later described it on the BBC as "spectacular". The following day he was due to chair Seminar I of the Congress; he immediately changed the agenda and put Nirenberg onto the main stage. He did this by using the time available for discussion but was careful to leave the sacrosanct coffee break intact [29].

3 | TOWARDS A TRIPLET CODE

Nirenberg and Matthaei's discovery changed everything – it broke with eight years of theorising about the genetic code and showed how the problem could be tackled experimentally. As is often the case in science, other people were also thinking along the same lines at the same time. In June that year, Peter Lengyel, a young researcher in Severo Ochoa's laboratory in New York, had come up with a very similar idea. When news of Nirenberg's breakthrough circulated on the grapevine at the beginning of August, the New York group immediately replicated the result. So at the same time as Nirenberg was stunning the audience in Moscow, Lengyel was showing that, in his hands too, some combination of U bases encoded phenylalanine [30].

Nirenberg's discovery triggered a frenzied race amongst US laboratories to discover more links between RNA nucleotide sequences and amino acids. In a reference to the shooting down in 1960 of an American U-2 spy plane over the Soviet Union, researcher Rollin Hotchkiss quipped that "The U-2 incident started the cold war, the U3 incident started the code war" [31]. Excited but unruffled, Crick returned to Cambridge and his experiment. But while Crick had been away, Brenner had not performed the various crosses that the pair had planned, and he was now about to go on a research trip to the Institut Pasteur in Paris. Crick was therefore left to his own devices with Leslie Barnett, but they were no longer simply looking for frame-shift mutations. The essence of the experiment that eventually congealed in the minds of Crick and Brenner in the late summer was that by combining mutations of various spins, it would be possible to demonstrate that the code was composed of combinations of three bases – three mutations of the same spin should restore the original function by putting the sequence back into the correct reading frame, whereas any other combination would not. This hypothesis-testing experimental objective, now often seen as the study's starting point, in reality emerged late on.

The task was substantial – Crick and Barnett had to map the effects of 80 different mutants (some 'spontaneous' – in reality induced by brief exposure to ultraviolet light – others induced by acridine dyes) and reveal the effects of combining some of these mutations in increasingly complex crosses. As they eventually described it, these mutations "were all suppressors of FC0, or suppressors of suppressors, or suppressors of suppressors of suppressors."[32] In what we now perceive as the key phase of the experiment, Crick and Barnett constructed

five triple mutants, five of the "+++" type, and one triple "negative" (remember, these signs were arbitrary), each of which behaved like FC0, exactly as their theory predicted.

Crick recalled the moment in late September when they observed the first mutant virus carrying triple suppressors:

> "And all we had to do was look at one plate. And see if it had any plaques on it. So we came in late at night, ten o'clock at night, or something, and there were plaques on the plate! So I said to Leslie, 'Let me check; we may have got the plates mixed up,' and she checked it, and then I told her, 'We're the only two to know it's a triplet code!"[33]

Strictly speaking, this was not true. Although the combination of mutations strongly suggested that the code was based on units of three bases, the experiments could not prove that to be the case – a code using groups of six bases was consistent with the results. This, however, would raise all sorts of problems by massively increasing the number of either meaningless or degenerate sequences (there would be 4096 possible combinations of bases, rather than a mere 64). As Crick later put it, this was "hardly likely to be taken seriously" [34].

Even before all the experiments were completed, Crick was telling colleagues around the world about the work. On 9 October, he wrote to Bob Sinsheimer at Caltech:

> "We now have convincing genetic evidence that the coding ratio is 3 or a multiple of 3. It is not clear that we can prove that it is 3 rather than 6, but we are trying" [35].

Crick thoroughly enjoyed this rare excursion into the laboratory. Odile, his wife, later recalled she had never seen him so cheerful. Crick put this down to the fact that the experiments seem to work so well, but other distractions may have helped [22]. As he wrote in his 1988 memoir *What Mad Pursuit*:

> "One evening, after dinner, I was working away in the lab when a glamorous friend of mine turned up and stood behind me while I continued to manipulate the tubes and plates. "Come to a party," she said, running her fingers through my hair. "I'm far too busy," I said, "but where is it?" "Well," she said, "we thought we'd hold it in *your* house." Eventually a compromise was reached. She and Odile would organise a small party and I would join them when I'd finished" [36].

4 | WRITING THE ARTICLE

At the beginning of November 1961, once all the careful experiments were completed, Crick wrote up the article, with editing input from Brenner (various versions of the manuscript are preserved) [37]. Crick was keen to ensure that proper credit was given to Nirenberg for his breakthrough, but given what he expected would be the significance of the experiment for studying the genetic code, he also wanted to demonstrate that he and Brenner had begun work on the project long before Nirenberg's discovery. He did this in two ways, publicly and privately. Publicly, he added a passage describing Nirenberg and Matthaei's discovery, almost as an afterthought, as the penultimate paragraph rather than as part of the background to the study:

> "At the recent Biochemical Congress at Moscow, the audience of Symposium I was startled by the announcement of Nirenberg that he and Matthaei had produced polyphenylananine (that is, a polypeptide all the residues of which are phenylalanine) but adding polyuridic acid (that is, an RNA the bases of which are all uracil) to a cell-free system which can synthesise protein. This implies that a sequence of uracils codes for phenylalanine, and our work suggests that it is probably a triplet of uracils" [38].

Privately, as soon as the manuscript was in the post, Crick sent two letters on the same day [38]. On 16 November he wrote to Raymond Latarjet, the organiser of the French conference he had attended in June, keen to ensure that his claim to priority would be backed up by the literature:

> "You may recollect that I reported our basic idea in the discussion (…) At the end of the conference I handed in a short written account. Could you tell me when and where it is likely to be published? It is the only simple means I have of establishing that we had the idea *before* Nirenberg's astonishing discovery" [39].

Crick also wrote to Nirenberg, including a copy of the article manuscript:

> "Dear Dr Nirenberg,
>
> I enclose an account of our genetical work which we have submitted to *Nature*. We had the basic idea in the summer, before your epoch-making discovery, and reported it at the Col de Voz DNA meeting in June, but we only got the triples after I returned from Moscow. Your PNAS papers arrived here the day before we sent off our MSS, so I was able to add the reference" [40].

Not all the results from the experiment were included in the paper. One of the reasons why some of the data were excluded might seem shocking to modern readers. They were not used because they did not make sense. They did not fit the theoretical explanation, they did not provide any insight, nor did they represent a coherent alternative.

Brenner and Crick referred to these infuriating, recalcitrant results as "barriers" – frameshifts that seemed to represent an "unacceptable" triplet. These could not be immediately explained by their overall theory, but they were confident they would one day give up their particular, uninformative secrets. Brenner later spoke frankly about this:

> "Now I have to tell you that there were exceptions. There were some things that didn't obey the rules. And a question one can ask is whether in science one should at least tell people about this. What we had was a huge body of information which was entirely self-consistent, but with a concept of the barriers. That is, certain frameshifts generated mutants themselves and therefore were not compatible. And the questions is what happens to all those exceptions? Well, you have the 'don't worry hypothesis' – there'll be an explanation for them. As it turned out it took about five more years to work through all the exceptions, and the remarkable thing is that each one of them had a different and special explanation. (…) So when one encounters something like this, it tells one that if one gets exceptions which cannot explain the coherent theory, the theory should remain. And it was wise of us to take all those exceptions, which showed no relationship amongst each other, and put them to one side. We didn't conceal them, we put them in an Appendix" [41].

That "appendix" was eventually published as a mammoth 73 page paper that appeared in 1967, including many more rII mutants that Brenner and Barnett had mapped in the meantime [42]. And indeed, all of the weird exceptions, barriers and all, were explained there in mind-numbing detail. Convinced that no one would actually read the article, given it was so long, turgid and detailed, Crick and Brenner attempted to smuggle a "personal communication" from Leonardo da Vinci into the article, but an eagle-eyed editor spotted the jape and it was removed [43]. The editor's comment in red pen has been preserved in manuscript, held in the Cold Spring Harbor Laboratory Archives and consultable online [44].

The paper appeared in *Nature* at the end of December 1961, signed by Crick, Brenner, Barnett and Watts-Tobin. As the title of the article indicated, unlike the work by Nirenberg, Ochoa and others, the results from the Cambridge group were solely focused on the *general* nature of the genetic code. They said nothing about its specific nature – there was no link between a particular DNA sequence and a given amino acid. Crick and Brenner were thinking on a far higher, more abstract level than any mere piece of biochemistry.

The article began with four fundamental conclusions, which were then explored and justified by a series of complex experiments, each of which contributed to the overall argument:

> "(a) A group of three bases … codes one amino acid.
>
> (b) The code is not of the overlapping type.
>
> (c) The sequence of the bases is read from a fixed starting point.
>
> (d) The code is probably 'degenerate'; that is, in general, one particular amino-acid can be coded by one of several triplets of bases" [46].

Strictly speaking, not all of these conclusions were proven in the subsequent five pages of data and dense argumentation. As the authors explained, it was technically possible that the number of bases in each group was six, or some other multiple of three. At some point in the writing of the article, a speculative paragraph attempting to explain their results in terms of a quintuplet code was deleted (Figure 2). This was probably a good idea. Second, although they did not have evidence that the code was "degenerate" this would "also account for the major dilemma of the coding problem, namely, that while the base composition of the DNA can be very different in different micro-organisms, the amino-acid composition of their proteins only changes by a moderate amount".

The article's conclusion was audacious, conveying the optimism that had swept through the scientific community after Nirenberg and Matthaei's transformation of the field:

> "If the coding ratio is indeed 3, as our results suggest, and if the code is the same throughout Nature, then the genetic code may well be solved within a year" [38].

The conditional "if the code is the same throughout Nature", a bet-hedging nuance, was added during the final stage of preparing the manuscript, apparently by Brenner (Figure 3). This optimistic view was shared by Nirenberg, who shortly afterwards predicted to Crick that "within another six months or so most of the genetic code will be cracked [45]."

Both men severely underestimated the difficulties ahead. Although progress was made in 1962, odd biochemical results soon led to a resurgence of theoretical explanations of the nature of the genetic code. These all proved both completely mistaken and profoundly distracting. As Crick put it in 1966, during this period there was "a flurry of theoretical papers, most of which are best forgotten" [46]. Eventually, hard-core biochemistry, in particular by Nirenberg and by Har Gobind Khorana, resolved the function of virtually all 64 codons and, in passing, also provided evidence that the codon was a triplet. In 1963, it was shown that oligodeoxynucleotides only four bases long were able to mobilise amino acids in cell-free systems; the codon was composed of three bases, not six. In 1967, the last of the 64 codons was deciphered, in a paper co-authored by Brenner and Crick [47]. This was the so-called opal codon, UGA. Appropriately enough, it read "stop".

5 | THE RECEPTION OF THE ARTICLE

Even before the article appeared at the end of 1961 (Figure 5), word got out as Crick sent letters and copies of the manuscript to his

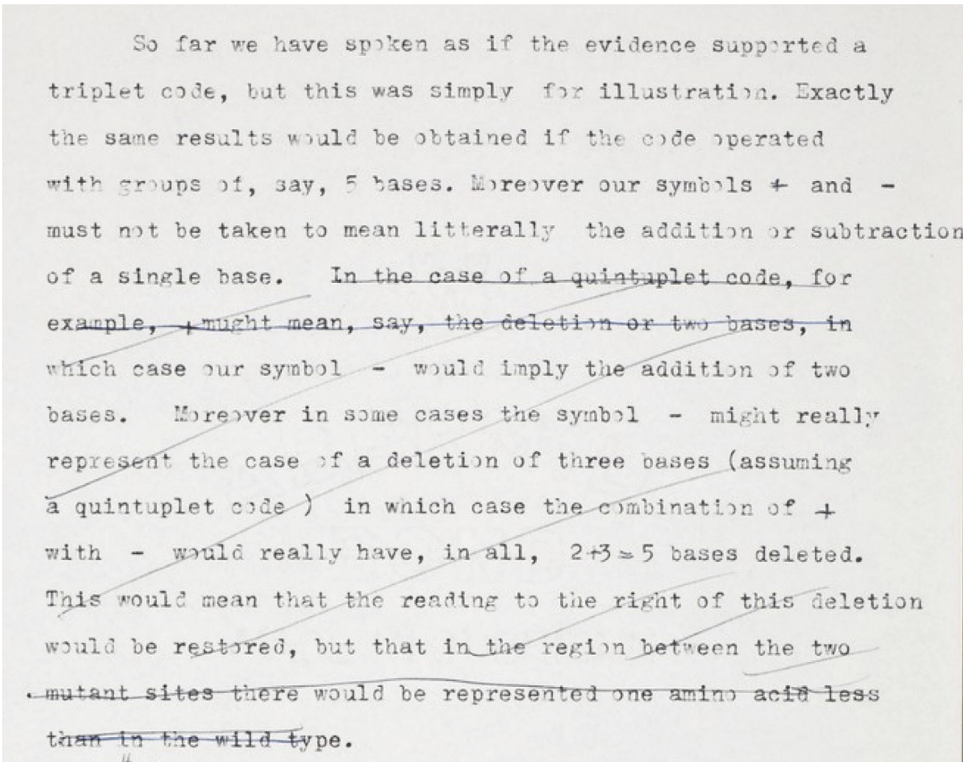
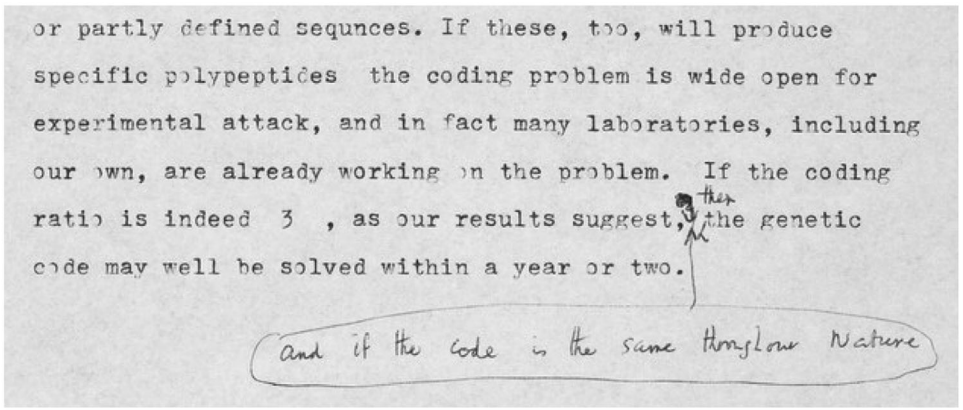

So far we have spoken as if the evidence supported a triplet code, but this was simply for illustration. Exactly the same results would be obtained if the code operated with groups of, say, 5 bases. Moreover our symbols + and - must not be taken to mean litterally the addition or subtraction of a single base. In the case of a quintuplet code, for example, + might mean, say, the deletion of two bases, in which case our symbol - would imply the addition of two bases. Moreover in some cases the symbol - might really represent the case of a deletion of three bases (assuming a quintuplet code) in which case the combination of + with - would really have, in all, 2+3=5 bases deleted. This would mean that the reading to the right of this deletion would be restored, but that in the region between the two mutant sites there would be represented one amino acid less than in the wild type.

FIGURE 2 Manuscript of the article, showing a deleted paragraph. CC CY-NC 4.0 The General Nature of the Genetic Code – Crick, et al – Nature. Wellcome Collection


or partly defined sequnces. If these, too, will produce specific polypeptides the coding problem is wide open for experimental attack, and in fact many laboratories, including our own, are already working on the problem. If the coding ratio is indeed 3 , as our results suggest, then the genetic code may well be solved within a year or two.

and if the code is the same throughout Nature

FIGURE 3 Final paragraph of the article, showing a manuscript correction, apparently in the hand of Sydney Brenner. CC CY-NC 4.0 The General Nature of the Genetic Code – Crick, et al – Nature. Wellcome Collection

correspondents around the world, and gave talks describing the experiments (Figure 4). In early December 1961, Crick gave a talk on the research at the Institut Pasteur in Paris. François Jacob was enthusiastic about what he heard, writing to Brenner on 13 December: "We had the visit of Francis who gave a remarkable seminar. This story really is astonishing" [48]. Others were not so convinced. On 27 November, Fritz Lippmann of the Rockefeller Institute wrote to Crick saying that he had read the manuscript but "without, to be frank, fully understanding the argument. I get the idea but I am rather slow and stupid in putting these genetic things together [49]." Lippmann was not only confused about the detail, he did not like the clear implication that there were 64 possible triplets but only 20 amino acids:

> "I am not too happy, however, with the idea of what you call a degenerate code. that is, assigning several triplets to one amino acid. (…) I have great trouble in imagining such a duplicity or maybe triplicity of coding for the same item."

Crick replied robustly:

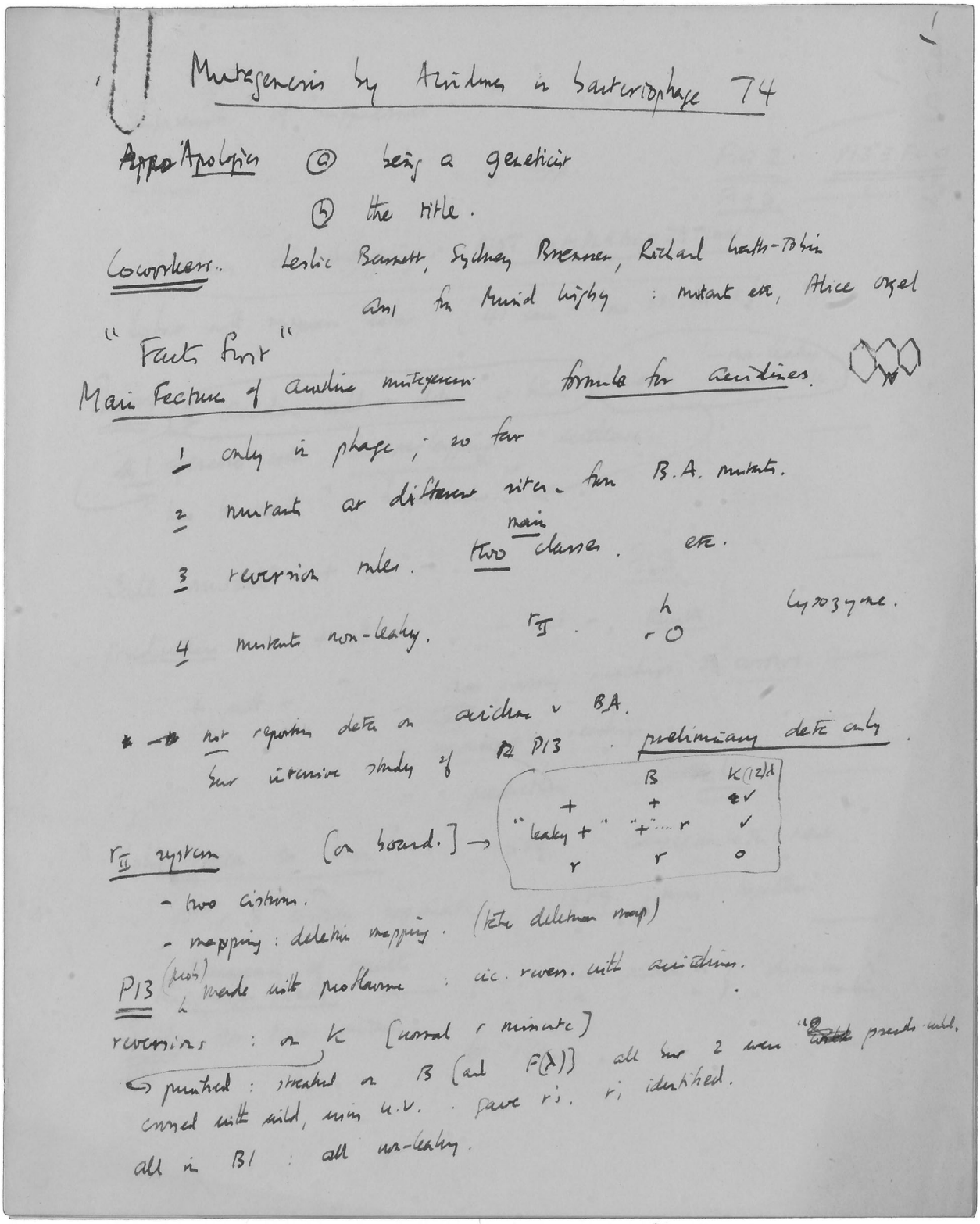


FIGURE 4 Crick's notes for a talk on the experiments described in the article, from the autumn of 1961. . CC BY-NC 4.0 'Mutagenesis by acridines in bacteriophage T4'. Wellcome Collection

FIGURE 5 Cover of the issue of *Nature* containing the Crick et al. article. Credit: Jeremy Norman's historyofscience.com

> "I am sure that the code is degenerate and it is not easy to explain even the present biochemical data without assuming this (…) However, Wittman's data[50] suggest that the code is not randomly degenerate – there is likely to be some connection between the various triplets which stand for one amino acid" [51].

(If there is such a consistent connection, it is still unknown six decades later…)

Brenner later explained why some contemporary readers were baffled by the article:

> "The other interesting thing about this was that it was a real 'house of cards' theory. You had to buy everything. You couldn't take one fact and let it stand by itself and say the rest could go. Everything was so interlocked. You had to buy the plus and minuses, you had to buy the barriers, you had to buy the triplet phase, and all these went together. It was the whole that explained it, and if you attacked any one part of it the whole thing fell apart. So it was an all or nothing theory. And it was very hard to communicate to people" [52].

Nirenberg's August announcement in Moscow and the furious spate of rival experimentation it unleashed sent a ripple of excitement through the scientific community. But oddly enough the media said nothing about the breakthrough. Eventually, that changed. On Christmas Eve 1961, the *New York Herald Tribune* announced "The code of life finally cracked", explaining the four-month press silence since Nirenberg's Moscow announcement by claiming that 'the news did not leak into the newspapers until last week'. A week later, the British *Sunday Times* took a similar line – "Scientists have cracked the code of life" – but chose to emphasise the role of British scientists and the importance of Crick's recent *Nature* paper. The next day, 1 January 1962, *The Times* heralded a "new stage in research on heredity", focusing initially on the Nirenberg experiment, but spent most of its column inches singing the praises of the Cambridge group. Even *The New York Times* joined in the Crick-fest, with an article on 2 February 1962 headlined "Hunter of Life's Secrets".

Crick was embarrassed by the coverage (as *The New York Times* accurately put it in its subtitle, "Francis Harry Compton Crick dislikes publicity") and wrote to Nirenberg to explain that he had done his best to set the record straight: "I have stressed that it is your discovery which was the real breakthrough" [53]. Nirenberg's response was typically relaxed. It also showed that the way in which the media treat scientific breakthroughs has not changed that much:

> "I haven't seen the English newspapers but the American press has been saying that this type of work may result in (1) the cure of cancer and allied diseases (2) the cause of cancer and the end of mankind, and (3) a better knowledge of the molecular structure of God. Well, it's all in a day's work" [49].

Pursued by the media, in January 1962, Crick gave a talk on the BBC, "Cracking the genetic code", in which he summarised the near-simultaneous breakthroughs by his group and that of Nirenberg [54]. A month later, Brenner gave his own talk, in which he explained the result in greater detail [55]. In what would turn out to be the run-up to the Nobel Prize for Crick, Watson and Wilkins' work on the double helix structure of DNA, Crick had been propelled to the centre of the media stage, no matter how uncomfortable he found it.

6 | CONCLUSION

Given how positively the article is now viewed, it is perhaps surprising that Crick was subsequently dismissive of its significance, pointing out that "it was pretty obvious it was *likely* to be a triplet code … the fact is, if we'd shown that the code was a *quadruplet* code, *that* would have been a discovery" [56]. He even suggested that "I think you could have deleted the whole work and the issue of the genetic code would not have been very different". The historian of molecular biology, Michel Morange, has taken a similar line, arguing that Crick and Brenner's "elegant experimental approach" "came too late" [57].

But all that is to look at the paper merely as part of the "race" to crack the genetic code. And indeed, although Crick was initially confident that frame-shifting mutants would "be very important for decoding" as he put it in that letter to Delbrück, that turned out not to be the case. The code was cracked by some hard biochemistry, not by the elegance of the arcane crosses of phage genetics. And yet the study was highly significant because it represented the epitome of the experimental and theoretical approach that Crick and Brenner had been developing in Cambridge, and that they would continue to practise over the subsequent decades. As an inspiration, a model, a challenge, this approach came at exactly the right time, as hundreds of researchers flocked to molecular genetics, while Crick and Brenner, like Benzer, Delbrück and others, prepared to leave it for the more challenging new shores of neuroscience.

Brenner later gave his explanation of why the underlying simplicity of the article's argument has been so attractive to readers down the decades:

> "This I think is the kind of apotheosis of a genetic analysis, because you have to consider what you're doing here: you're taking these viruses and you are just mixing them together and you are simply recording plus and minus. And from this pattern it seems mad that you could deduce the actual triple nature of the genetic code. But this is simply the logic of how the genetic information is transferred – it's a non-overlapping triplet code" [58].

Crick, who for a man who was supposedly never in a modest mood could be remarkably self-effacing, breezily unravelled the process involved in the experimentation and writing of the article in an interview with the historian Horace Judson:

> "It's the usual business – it seems very straightforward, and when you actually look what happened, you did it for a lot of *silly* reasons that led you to the right thing" [59].

That is true, and it highlights the deceptive structure of a great scientific article such as the one published by Crick and Brenner in *Nature*, which, by its argument and presentation of data, seduces the reader into accepting that is how things really were, even if they were not. But Crick's modesty hides the exciting and unique process of trying to understand the results, of staring at data and sensing that there is an underlying order and meaning that can be apprehended. That feeling, which all scientists have known – if not with such significant data – is one of the immense intellectual attractions of the scientific endeavour. Brenner later described this idyllic experience in lyrical, heartfelt terms, underlining the significance of the article 60 years on, and beyond:

> "this was one of the most beautiful, aesthetically elegant experiences of my life, in which just by doing these little operations you landed up with this detailed description of the molecular structure of living matter" [59].

DATA AVAILABILITY STATEMENT

The data that support this paper are available at the Wellcome Trust Francis Crick Archives: https://wellcomecollection.org/works/hz43r7re

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